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TISSUE UBIQUINONE AND VITAMIN E LEVELS IN RATS WITH EXPERIMENTAL FOCAL MYOCARDITIS AND HYPOXIC HYPOXIA

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UDC 616.127-002-031.84+616-001.8]-092.9-07: 616-008.93:[577.161.3+577.161.6]-074

KEY WORDS: ubiquinone; vitamin E; hypoxia; focal myocarditis.

Vitamin E not only acts as a biological antioxidant, but it also plays the role of regulator of energy metabolism [1]. Vitamin E and ubiquinine occupy a special place in the normalization of metabolism in the myocardium [9, 10]. In diseases accompanied by disturbance of the circulation, the content of vitamin E and ubiquinone in the myocardium changes [4, 9]. Data on the concentration of these substances in the tissues in various states accompanied by hypoxia are conflicting [8, 12], and this makes it difficult to understand the mechanism of their action.

The object of this investigation was to study the concentrations of vitamin E and ubiquinone in different tissues of rats with acute hypoxic hypoxia and with histotoxic hypoxia arising after administration of adrenalin [6].

EXPERIMENTAL METHOD

Experiments were carried out on inbred male albino rats weighing 160-180 g. Focal myocarditis was studied 24 h after intramuscular injection of 0.4 ml of a 0.1% solution of adrenalin hydrochloride during the period of maximal morphological changes in the myocardium [3]. Hypoxic hypoxia was induced by taking the rats gradually in a pressure chamber to an altitude of 10,000 m (pressure 200 mm Hg). The animals were decapitated. Tissues were removed and homogenates and mitochondria obtained in the cold. Mitochondria were isolated from heart muscle in 0.25 M sucrose made up in 0.05 M Tris-HCl buffer with 0.001 M EDTA. The concentrations of ubiquinone and vitamin E were determined as described previously [2] and protein by Lowry's method [11]. The results were subjected to statistical analysis by Oivin's method [5].

EXPERIMENTAL RESULTS

The concentration of vitamin E in the kidneys of the animals with experimental focal myocarditis was reduced by 44.7%. By contrast, the vitamin E concentration in the myocardium showed a tendency to rise (Table 1).

The ubiquinone concentration in the myocardial tissue was increased by 35.3%, whereas at the same time in the liver tissue it was reduced by 50.7% (Table 1). Changes in the ubiquinone and vitamin E concentrations in the various tissues were in the same direction.

Department of Coenzyme Biochemistry, A. V. Palladin Institute of Biochemistry, Academy of Sciences of the Ukrainian SSR, Kiev. (Presented by Academician of the Academy of Medical Sciences of the USSR N. N. Gorev.) Translated from Byulletin' Éksperimental'noi Biologii i Meditsiny, Vol. 94, No. 9, pp. 36-38, September, 1982. Original article submitted February 19, 1982.

0007-4888/82/9409-1203$07.50

TABLE 1. Concentrations of Ubiquinone and Vitamin E (in μg/g protein) in Tissues of Intact Rats (control) and Rats with Focal Myocarditis (M ± m)

Test object	Ubiquinone		Vitamin E	
	control	experiment	control	experiment
Heart	680±77	920±75*	840±79	940±73
Mitochondria of heart	4480±390	6940±780*	1850±350	4080±600*
Liver	730±65	370±57 †	340±61	270±41
Muscles	76±13	58±6,5	96±17	67±9,0
Kidneys	520±66	370±31	390±88	270±11*

*P < 0.02 compared with control.
†P < 0.01 compared with control.

TABLE 2. Concentrations of Ubiquinone and Vitamin E (μg/g protein) in Tissues of Intact Rats (control) and of Rats with Hypoxic Hypoxia (M ± m)

Test object	Ubiquinone		Vitamin E	
	control	experiment	control	experiment
Heart	730±50	750±64	780±75	1100±41*
Mitochondria of heart	2600±146	4130±485*	6700±1300	10300±1660
Liver	620±68	610±53	390±44	290±12
Muscles	54±5	71±16	110±23	66±6,5
Kidneys	890±50	470±40	410±46	310±35

*P < 0.01 compared with control.

In experimental myocarditis after injection of adrenalin foci of necrosis are formed in the myocardium, causing nutritional changes, disturbances of contractility, and the development of histotoxic hypoxia [6]. The primary disturbance is that of energy metabolism [3]. Accordingly the rise in the ubiquinone concentration in the myocardium may be compensatory, aimed at activating tissue respiration. Since oxidative phosphorylation processes take place in the mitochondria, where most of the ubiquinone and vitamin E are concentrated, the content of these components was determined in mitochondria isolated from the myocardium.

In myocarditis the vitamin E concentration in the mitochondria was increased by 122% and the ubiquinone by 56.8% (Table 1). This could perhaps be connected with their intracellular redistribution.

In rats with experimental focal myocarditis a compensatory and adaptive increase in the concentrations of ubiquinone and vitamin E was thus observed in the myocardial mitochondria.

In hypoxic hypoxia, after the rats had been taken up to a conventional altitude of 10,000 m (Table 2), an increase in the vitamin E concentration was observed in the myocardium. The ubiquinone concentration was virtually unchanged in all the tissues studied. The possibility cannot be ruled out that changes observed in the vitamin E concentration on the various tissues of the hypoxic rats are connected with a redistribution of vitamin E among the tissues.

The ubiquinone concentration in the myocardial mitochondria was increased (Table 2). Since the ubiquinone level in the myocardial tissue was virtually unchanged, the increase in its concentration in the mitochondrial fraction probably takes place on account of intracellular redistribution.

Vitamin E is known to have a protective effect in various hypoxic states [7], and for that reason the increase in its concentration in the mitochondrial fraction of the myocardium of rats with myocarditis and the tendency for it to increase in hypoxic hypoxia were rather unexpected. However, the study of the distribution of vitamin E in the tissues showed that this may be connected with a decrease in its concentration in other organs, and that it may be compensatory. Among the tissues studied in rats with hypoxia and adrenalin-induced myocarditis, the myocardium, it will be noted, showed the most marked changes in the concentrations of vitamin E and ubiquinone in both magnitude and direction. This could be evidence of the greater compensatory powers of the myocardium and the special role of this organ in maintaining the resistance of the body to these pathogenic factors.

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ROLE OF FOLIC ACID AND ITS AMINO-DERIVATIVES IN THE MECHANISM OF ACTION OF LOW DOSES OF FORMALDEHYDE ON ORGANS

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UDC 615.361.014:41:547.281.1].015.42:612.015.6:577.164.17

KEY WORDS: formaldehyde; folic acid; kidneys; rats.

Close metabolic relations are known to exist between formaldehyde and folic acid [1, 8, 9].

Considering the protective effect of low doses of formaldehyde on organs in acute ischemia [2, 4, 6, 7] and the fact that it has been used successfully for conserving organs and tissues [3, 5], it was decided to study relations between the concentration of folic acid and its derivatives and the quantity of formaldehyde administered to an animal with a view to conserving the heart, liver, and kidneys.

EXPERIMENTAL METHOD

Experiments were carried out on 58 noninbred rats of both sexes weighing from 150 to 250 g. Before receiving formaldehyde the animals were given folic acid or aminopterin or methotrexate* by intramuscular injection in a dose of 1 mg on three consecutive days. Next, 0.1% formalin in Ringer's solution was injected in a dose of 5 ml (55 μM formaldehyde) into the inferior vena cava. The heart, liver, and kidneys were removed from the animal immediately after sacrifice and the quantity of formaldehyde in them was determined. Experiments in which the same quantity of formaldehyde was injected under identical conditions, but without preliminary administration of pterins served as the control.

The concentration of formaldehyde also was determined in 32 experiments *in vitro* after incubation of equal volumes of formaldehyde (25 μM), folic acid (0.5 μM), or aminopterin or methotrexate (0.5 μM) for 10 min at 20°C.

The formaldehyde concentration was determined in protein-free extract or solution by a method based on specific interaction between formaldehyde and chromotropic acid in the presence of sulfuric acid, with the development of a violet color. The sensitivity of the method is $\geqslant 0.1$ μmole/g tissue.

EXPERIMENTAL RESULTS

The experiments showed that folic acid, and also aminopterin and methotrexate, removed more than 50% of formaldehyde from the solution, reducing its concentration from 23.20 ± 0.38 μmoles/ml in the control to 10.75 ± 0.21 μmoles/ml ($P < 0.01$), 9.42 ± 0.58 μmoles/ml ($P < 0.01$), and 8.68 ± 0.98 μmoles/ml ($P < 0.01$) respectively after the addition of the above-named compounds.

*The authors are grateful to Doctor of Biological Sciences N. A. Andreeva for advice and for generously providing the preparations.

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